SECTION ON MICROBIOLOGY

FEBRUARY 18, 1948

I. EXECUTIVE SESSION
Reading of the Minutes.

II. PAPERS OF THE EVENING

BACTERIAL TOXINS

a. The preparation and properties of purified toxins and toxoids.
Louis Pillemer, Ph.D.
Western Reserve University Medical School, Cleveland

b. The iron enzymes of C. diphtheriae and their possible relation to diphtheria toxin
Alvin M. Pappenheimer, Jr., Ph.D.
New York University College of Medicine

c. Current status of immunization against diphtheria
Donald T. Fraser
University of Toronto, Toronto, Canada

d. The immunization of adults with diphtheria toxoid
H. Sherwood Lawrence
Third Medical Division, Bellevue Hospital

III. DISCUSSION
Gregory Shwartzman, *Chairman*
Harry Most, *Secretary*

The Preparation and Properties of Purified Toxins and Toxoids

LOUIS PILLEMER, Ph.D.

From the Institute of Pathology, Western Reserve University, Cleveland, Ohio

Chemical investigations on bacterial toxins and toxoids are of practical and theoretical significance not only in the field of immunochemistry but also in the related preclinical and clinical sciences. The ultimate aim of such studies is to determine the nature, the character, the relationship between the biological and chemical properties, and the mode of action of toxins and toxoids.

The proper approach to these problems should concern first the determination of the physical, chemical and immunological properties of toxins and toxoids. In order to accomplish this, isolation of these substances in pure form is necessary. It is the purpose of this paper to discuss recent efforts to purify and characterize tetanal toxin and toxoid and diphtherial toxoid.

Both Eaton and Pappenheimer have prepared diphtherial toxin in a state of high purity. Groups of Army and Navy personnel at Camp Detrick have crystallized botulinal toxin (Type A). These investigators employed the classical methods of protein purification which involve the use of neutral salt precipitation, acid precipitation and adsorption. These procedures are largely based on the work of Denis and others which was carried out approximately a century ago, long before the chemical nature of proteins was understood. Although such empirical methods have yielded satisfactory preparations, it appears that these procedures leave much to be desired and that the problem of purifying toxins and toxoids should be solved by the employment of

precise methods which conform to the disciplines of physical chemistry.

Using the basic principles developed by Cohn and his associates for the fractionation of blood plasma in ethanol-water mixtures at low salt concentrations and low temperatures, the author and his colleagues have prepared crystalline tetanal toxin and highly purified staphylococcal and pertussal toxins. Pure diphtherial and tetanal toxoids have also been prepared. The methods employed involve the use of methanol-water mixtures under precisely controlled conditions of pH, ionic strength and temperature and have been reported in detail elsewhere.

Crystalline tetanal toxin has been characterized as a heat labile protein with an isoelectric point of 5.1 ± 0.1. The toxin has an electrophoretic mobility of 2.8 x 10^{-5} in veronal buffer of 0.1 ionic strength at pH 8.6 and has a sedimentation constant of 4.5 Svedberg units. Freshly prepared toxin has substantially constant solubility. The crystalline toxin gives the usual protein reactions, and contains 1 per cent sulphur and traces of phosphorus. It does not contain carbohydrate. The crystalline protein does not precipitate anti-*Clostridium tetani* rabbit serum. Four times recrystallized tetanal toxin contains between 3400 and 3600 flocculating units and about 6.6 x 10^7 mouse minimal lethal doses per mg. of N. Crystalline tetanal toxin spontaneously converts to a flocculating atoxic dimer upon standing at 0° C. This change is accompanied by the appearance of another molecular species judged both by constant solubility test and ultracentrifugal analysis. The flocculating atoxic dimer has a sedimentation constant of 7 Svedberg units.

Pure diphtherial toxoid has been separated as a heat labile protein with an electrophoretic mobility of 8.1 x 10^{-5} in veronal buffer of 0.1 ionic strength at pH 8.6 and with a sedimentation constant of 4.6 Svedberg units. Its isoelectric point is 4.7 ± 0.1. The preparation has constant solubility and satisfies the existing criteria for a pure protein. It does not contain sulphur, phosphorus, carbohydrate or iron. The purified diphtherial toxoid does not precipitate anti-*Corynebacterium diphtheriae* rabbit serum. The final product contains between 2000 and 2200 flocculating units per mg. of N.

Purified diphtherial and tetanal toxoids obtained by the low temperature methanol method are now commercially available. They possess a number of practical advantages which may be summarized as follows. These toxoids which are more than 200 times purer than crude preparations generally do not elicit side-reactions when administered to children and adults. In general, it is no longer necessary to carry out reactor tests, and "booster" doses may be given without hesitation. The immunity obtained with these products is durable and of high order. The immunizing power of toxoids is potentiated by alum precipitation but unfortunately alum occasionally produces local irritation at the site of injection. Since the new preparations contain fewer solids, they can be precipitated with one-tenth the amount of alum required for crude toxoids. Side reactions to alum are therefore minimized. The addition of glycine permits sterilization by filtration and allows storage of these preparations for long periods of time under extreme temperature conditions without deterioration. Finally, since these toxoids are pure and stable they can serve as primary standards for the biological evaluation of products of equal or lesser purity.

The above mentioned advantages should lead to an increased use of toxoids for the prevention of diphtheria and tetanus. It should also lead to an intensified effort to purify by similar methods other toxins, toxoids and bacterial antigens.